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Endocannabinoids and Their Role in Fatty Liver Disease

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Abstract
The endocannabinoid system comprises receptors, CB1 and CB2, their endogenous lipidic ligands and machinery dedicated to endocannabinoid synthesis and degradation. An overactive endocannabinoid system appears to contribute to the pathogenesis of several diseases, including liver diseases. With the increasing incidence of non-alcoholic fatty liver disease (NAFLD) in parallel with the obesity epidemic, the development of effective therapies is gaining considerable interest. Several recent experimental lines of evidence identify CB receptors as potential novel therapeutic targets in the management of NAFLD. Endogenous activation of peripheral CB1 receptors is a key mediator of insulin resistance and enhances liver lipogenesis in experimental models of NAFLD. Moreover, we have shown that adipose tissue CB2 receptors are markedly upregulated and promote fat inflammation, thereby contributing to insulin resistance and liver steatosis. Data from our group also indicate that tonic activation of CB1 receptors is responsible for progression of liver fibrosis, whereas CB2 receptors display anti-fibrogenic properties. The clinical relevance of these findings is supported by studies in patients with chronic hepatitis C indicating that daily cannabis use is an independent predictor of both fibrosis and steatosis severity. Moreover, preliminary data derived from clinical trials strongly suggest that selective CB1 antagonism improves insulin resistance and reduces liver fat. Tempering these promises, the first generation of CB1 antagonists raised concern due to an alarming rate of mood disorders and the development program of these molecules was suspended. Current research efforts are therefore focused on developing formulations of CB1 antagonists that do not enter the central nervous system, and preliminary experimental data obtained with such molecules are encouraging.

Introduction

The prevalence of obesity and the metabolic syndrome is on the rise. As a consequence, NAFLD, the hepatic hallmark of the metabolic syndrome, has become a common finding in clinical practice with a prevalence of 20–30% in Western countries [1, 2]. The spectrum of the disease ranges from simple steatosis, a condition generally associated with a benign liver outcome, to steatohepatitis, an entity that associates with steatosis, liver inflammation and hepatocellular injury. The latter stage is associated with an activation of fibrogenic pathways and carries a

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10–20% risk of cirrhosis after 10–20 years [3]. As underscored in several recent studies, NASH is entailed with increased liver-related mortality due to end-stage liver disease or development of hepatocellular carcinoma [4]. Management of NAFLD remains a major challenge as no treatment has been approved for this indication as yet. Therapeutic strategies currently aim to decrease insulin resistance as well as the processes leading to necroinflammation and hepatic fibrosis. In this context, modulation of cannabinoid receptors is emerging as a potential novel therapeutic approach. These receptors are part of a novel signaling pathway, known as the endocannabinoid system, that is increasingly incriminated in a variety of physiological and pathological conditions.

The Endocannabinoid System

Preparations of the hemp plant *Cannabis sativa* have been used for medicinal purposes over centuries. Δ9-tetrahydrocannainol (THC) was identified in 1964 as the predominant cannabinoid compound responsible for the psychoactive effects of marijuana. Two decades later, CB1 and CB2 cannabinoid receptors were identified as receptors for phytocannabinoids, paving the way for the characterization of the endocannabinoid system that now comprises CB receptors, bioactive lipidic endocannabinoid ligands and enzymes involved in their biosynthetic and degradative pathways [5, 6].

CB1 and CB2 receptors are 44% identical and belong to the G protein-coupled receptor family. CB1 receptors are expressed at high levels in the brain and at lower levels in a large number of peripheral tissues [5, 6]. These receptors are exclusively responsible for the central psychotropic and behavioral effects of cannabinoids and are also engaged in a wide variety of peripheral functions, such as the control of energy homeostasis, cardiovascular function and reproduction, etc. [6–8]. In contrast, CB2 receptors are mainly expressed in the periphery, predominantly by immune cells, and play a key role in inflammatory processes [9, 10]. In addition, several studies have shown that endocannabinoids may also bind other receptors and stimulate nonreceptor-mediated pathways. Cannabinoid receptors signal through Gi/o-dependent pathways, eliciting inhibition of adenylyl cyclase, stimulation of MAP kinases and PI3 kinase, and CB1-dependent modulation of calcium and potassium channels. Moreover, G protein-independent pathways have also been identified, including ceramide production and COX-2 induction [11].

Ligands for CB receptors include phytocannabinoids and fatty acid-derived endocannabinoids with predominantly autocrine/paracrine effects, owing to their lipophilic properties [5–7, 12]. Δ9-tetrahydrocannainol (THC), the major psychoactive ingredient of *C. sativa*, binds CB1 and CB2 receptors with similar affinity [13]. Among endocannabinoids, anandamide (arachidonoyl ethanolamide) and 2-arachidonoyl glycerol (2-AG) are the two best studied. Anandamide is a major CB1 ligand and shows low affinity for CB2 receptors, whereas 2-AG is a full agonist for both CB1 and CB2 receptors. Both compounds are synthesized from distinct membrane phospholipid precursors via parallel pathways involving phospholipase D for anandamide and diacylglycerol lipase for 2-AG. Following receptor binding, arachidonoyl ethanolamide and 2-AG rapidly undergo cellular reuptake by a specific transporter prior to degradation by fatty acid amide hydrolase and monoacylglycerol lipase, respectively. Lipid mediators other than anandamide and 2-AG have been reported to bind CB receptors, but their biological significance remains undetermined [14, 15]. Synthesis of endocannabinoids is immediately followed by release, so that in the absence of storage, tissue levels vary according to their rate of synthesis and degradation [7].

Pharmacological modulators of cannabinoid receptors and mouse strains deficient in these receptors have been key tools in uncovering a growing list of functions regulated by endocannabinoids in physiological and pathological conditions. As a result, a growing body of literature that indicates an overactive endocannabinoid system is a critical mediator in various acute and liver conditions, such as cirrhotic portal hypertension, metabolic and ethanol-induced fatty liver, ischemia reperfusion and the scarring process associated with chronic liver disease, has recently accumulated [16–22].

Modulators of CB Receptors for Pharmacotherapy: Hopes and Concerns

Rimonabant (Acomplia®, Sanofi-Aventis) was the first selective CB1 ligand introduced into clinical practice. As an antagonist/inverse agonist, the compound can affect constitutive endocannabinoid signaling in the absence of CB1 receptor stimulation [23]. The molecule was initially developed for the management of obesity [24–27] because of its ability to reduce central orexigenic effects of endocannabinoids and its positive impact on body weight in preclinical studies. It soon became clear that CB1 antago-

nism also ameliorates obesity-associated dyslipidemia, waist circumference and insulin resistance to a greater extent than what would have been expected from weight loss alone [24–27]. In keeping with clinical data, parallel laboratory studies uncovered significant peripheral effects of CB1 antagonism on features of the metabolic syndrome associated to obesity. Thus, treatment of HFD mice with rimonabant increases adipose tissue energy expenditure and lipolysis [28]. In addition, rimonabant stimulates glucose uptake both in skeletal muscles [29, 30] and adipose tissue [31], and increases fat synthesis of adiponectin [32, 33], thereby improving insulin resistance. Altogether, these observations led to further trials assessing the impact of the molecule on diabetes, dyslipidemia and cardiovascular risk in obese patients [34–37]. In parallel, rimonabant and other CB1 receptor/antagonists were assayed for their benefit on alcohol dependence, tobacco withdrawal and in neurodegenerative disorders [7]. Unfortunately, these exciting advances were rapidly clouded by the alarming incidence of central side effects, which included nausea, anxiety, sleep disorders and depression. Considering the unfavorable balance of adverse effects with respect to benefits in obese patients, the European Medicine Agency recommended marketing withdrawal of the drug in October 2008, and ongoing clinical development of other CB1 antagonists/inverse agonists not yet approved was halted around the same time [23].

Modulation of CB2 receptors is also emerging as a potential therapeutic strategy, predominantly in inflammatory conditions such as pain, atherosclerosis and allergies [7, 38]. However, ligands of CB2 receptors have not reached a clinical stage as yet. Nevertheless, these compounds are devoid of central side effects and, therefore, are of growing interest.

Beneficial Effects of Cannabinoid Receptor Antagonism in NAFLD

CB1 Receptors Promote Metabolic Steatosis and Insulin Resistance

Recent experimental and clinical studies indicate that de novo liver fat synthesis accounts for a significant source of triglycerides in steatotic livers [39, 40]. This enhancement of liver lipogenesis has been linked to an overactivation of the CB1 tone in obese mice, with an induction of CB1 receptors in hepatocytes and an increase in liver levels of anandamide [19, 41]. Conversely, genetically obese fa/fa rats treated with rimonabant show reversal of hepatic steatosis and improved insulin sensitivity [32]. In order to determine the specific role of the hepatocyte CB1 receptor, experiments have been conducted in mice bearing a selective deletion of CB1 receptors in hepatocytes. Interestingly, these animals are protected from diet-induced steatosis and insulin resistance, although they do become obese [20]. Finally, studies in cultured hepatocytes indicate that upregulated CB1 receptors directly enhance lipid accumulation in hepatocytes by stimulating lipogenic pathways and inhibiting fatty acid β-oxidation [19, 20, 42]. Collectively, these data indicate that peripheral overactivation of CB1 receptors promotes obesity-associated fatty liver and insulin resistance. Aside from its contribution to steatogenesis, CB1-dependent endogenous cannabinoid tone may also favor the inflammatory response associated with NASH. Thus, it has been shown that endogenous CB1 activation reduces secretion of adiponectin [33], an adipocytokine with potent anti-inflammatory effects in the liver [43]. In keeping with these observations, administration of rimonabant to genetically obese rats induces a significant improvement in the hepatic inflammatory response [32].

Data from epidemiological and therapeutic studies also support the causal role of cannabinoids in the pathogenesis of steatosis. Thus, in patients with chronic hepatitis C, daily cannabis use has been identified as an independent predictor of the presence of severe steatosis [44]. Moreover, analysis of data pooled over one year from four pivotal trials in overweight patients showed a significant decrease in alanine aminotransferase in patients under rimonabant [27]. The ADAGIO trial in 800 patients with abdominal obesity and dyslipidemia recently reported similar findings [37]. Interestingly, a computed tomography substudy evaluated the distribution of fat depots and demonstrated for the first time in humans that rimonabant induces preferential loss of visceral fat over subcutaneous fat and reduces liver steatosis. Overall, these results provide convergent evidences for a steatogenic role of CB1 receptors in patients with NAFLD.

Pro-Inflammatory Effects of CB2 Receptors in Fat Tissue Participate in the Pathogenesis of NAFLD

It is well established that low-grade adipose tissue inflammation associated with obesity contributes to insulin resistance and NAFLD [43, 45]. Since CB2 receptors are potent regulators of innate immunity [9], we investigated their potential role in the pathogenesis of obesity-associated NAFLD. We found that in obese mice, CB2 receptors are markedly upregulated in the visceral adipose tissue and promote fat inflammation [46]. More-

over, treatment with the CB2 agonist JWH-133 enhances insulin resistance and steatosis in these animals; in contrast, CB2-deficient animals show improved glucose tolerance and resistance to fatty liver [46]. Collectively, these preclinical data indicate that CB2-induced fat inflammation contributes to the pathogenesis of obesity-associated insulin resistance and fatty liver. The role of CB2 receptors in obese patients remains to be investigated. Interestingly, it has been shown that circulating levels and adipose tissue production of 2-AG are elevated in obese patients [47, 48], suggesting that the endogenous CB2 tone is also enhanced in obese patients.

CB Receptors Regulate Liver Fibrogenesis and Portal Hypertension

Activation of fibrogenic pathways is a critical mechanism associated with the transition from isolated steatosis to steatohepatitis. Studies from our lab and other groups strongly suggest a major impact of the endocannabinoid system in the regulation of liver fibrogenesis. Indeed, we found that CB1 and CB2 receptors are markedly upregulated in cirrhotic liver samples, primarily in hepatic myofibroblasts, and demonstrated that endogenous activation of CB1 receptors enhances fibrogenesis. Conversely, stimulation of CB2 receptors counteracts progression of fibrosis [21, 22].

Anti-fibrogenic properties of CB2 receptors were established using the CCl4 model, based on the findings that CB2-deficient mice show enhanced survival of liver fibrogenic cells resulting in increased fibrosis [22]. In keeping with our findings, a subsequent study in rats with established cirrhosis showed that administration of the CB2 agonist JWH-133 improves liver fibrosis, decreases the inflammatory infiltrate and reduces the density of hepatic myofibroblasts following increased apoptosis [49]. Overall, these data strongly suggest that selective CB2 agonists may prove useful in the management of hepatic fibrosis.

The role of CB1 receptors in liver fibrosis was examined in models of carbon tetrachloride or thioacetamide intoxication and in bile duct-ligated animals: administration of rimonabant to wild-type mice or genetic inactivation of CB1 receptors were both associated with a significant reduction in fibrosis progression [21]. Pro-fibrogenic properties of CB1 receptors were ascribed to the presence of upregulated CB1 receptors in hepatic myofibroblasts, resulting in a combined mitogenic and anti-apoptotic effect. Recently the anti-fibrotic potential of CB1 antagonism was also documented in a murine model of prolonged (9.5 months) high-fat feeding that recapitulates histological features of NASH, including steatosis, foci of inflammatory cells and significant fibrosis [50]. In these animals, administration of rimonabant after the onset of steatohepatitis reduced progression of fibrosis, as compared to control littermates.

We further evaluated the clinical relevance of these experimental findings by investigating the impact of cannabis use on fibrosis severity in 270 individuals infected with hepatitis C virus. Daily cannabis consumption over the course of infection was a strong independent predictor of fibrosis severity, suggesting that CB1 signaling dominates over CB2 during chronic hepatitis C [51]. Similar findings were reported in an independent cohort of patients [52].

Conclusion – Future Perspectives

Overall, there are overwhelming clinical and animal data supporting a major role of endocannabinoids in the pathogenesis of several features of NAFLD. Tempering these promises, concern for psychiatric safety of CB1 antagonists has unfortunately put an end to the clinical development of antagonists/inverse agonists that enter the brain. Nevertheless, considering the meaningful clinical benefits expected from therapeutic developments in liver diseases and other fields, research efforts should be pursued, and the challenge will be to design newer and more specific formulations such as neutral CB1 antagonists or molecules unable to cross the blood-brain barrier that might retain therapeutic properties without exposing patients to central side effects. Preliminary results recently reported support this approach with a novel peripherally restricted neutral CB1 receptor AM6545. Indeed, in obese mice, the compound elicits a sustained decrease in hepatic steatosis and an improvement in glucose tolerance, and shows no detectable effect on the central nervous system [53].

Disclosure Statement

The authors declare that no financial or other conflict of interest exists in relation to the content of the article.

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